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(54) STREPTOCOCCAL ANTIGEN, PHARMACEUTICAL COMPOSITIONS CONTAINING IT AND ITS USE IN MEDICAL DIAGNOSIS AND TREATMENT

(71) We, MEMM S.p.A., of 35 Via Farini, Bologna, Italy, an Italian Body Corporate, do hereby declare the invention, for which we pray that a patent may be granted to us, and the method by which it is to be performed, to be particularly described in and by the following statement;-

The present invention relates to the diagnosis and treatment of neoplasms. More particularly, it relates to a certain newly discovered bacterium, of the genus *Streptococcus*, which permits the immunological system of the host to signal the presence or absence of a neoplasm and which can be used also to eliminate the neoplasm. The invention also relates to compositions containing this bacterium, to active extracts of the bacterium and to the use of the bacterium, the compositions and the extracts in the diagnosis and treatment of neoplasms.

Many studies have been conducted which show that cancer cells contain cancer-specific antigen or antigens. For example, studies have indicated that all cancers induced by a given virus in any single strain of susceptible rodents show group specificity, i.e. they all have the same antigen. On the other hand, individual specificity has been shown for spontaneous and chemically-induced cancers. These studies have also shown that such cancers have different antigenic determinants, even though induced by the same chemical, and that each of multiple cancers induced in one animal by the same chemical also has a specific antigenicity.

However, the possibility has never been ruled out that there is at least one antigen which is common to all neoplastic cells. In the book "Immunological Surveillance" by Sir MacFairlane Burnet, Pergamon Press 1970, page 13, it is stated:

"In the last analysis, any theoretical disinclination to consider the possibility of an antigen being present in all tumours as an essential part of the malignant process would be effectively countered by a single fully acceptable experimental demonstration."

Another factor which has been recognized by some researchers is the existence of a so-called "blocking factor", which blocks the inhibition of cancer colony formation by lymphocytes. Anderson ["Immunotherapy of Cancer", appearing in "Recent Advances in Cancer and Radiotherapeutics: Clinical Oncology" edited by Halnan, The Williams and Wilkins Company (1972) pages 200 - 201] points out that there is strong support for the theory of the existence of antibody-like materials which block receptors on the tumour cells so that antigen-reactive lymphocytes cannot recognize and attack the cells. In discussing these observations, Anderston states at page 201:

"Hosts in which cancer grew had factors in their sera which blocked inhibition of their own cancers' colony formation by their own lymphocytes, presumably by combining with or coding the cancer cells".

We have now discovered that certain species of the genus *Streptococcus* have the ability to produce an antigen which can be used in a serum agglutination test for the presence or absence of neoplasms in a patient to whom cancer is suspected. Furthermore, the new antigen or bacterial cells containing it can be used in such a manner as to permit the natural

immunological system of the host to attack and destroy such neoplasms.

Thus, the present invention provides a species of the genus *Strepococcus* capable of producing an antigen which is characterized by the property of causing agglutination of sera of patients free from neoplasms whilst not causing agglutination of the sera of neoplastic patients.

The ability of any particular species of *Streptococcus* to produce such an antigen may easily be determined by a simple agglutination test, well-known to those skilled in the art. A particular species which has been found capable of producing such an antigen is *Steptococcus faecalis subspecies G* which has been isolated from a sample of air collected near Modica, Italy, and which has been deposited in the American Type Culture Collection under the accession No. ATCC 31,290. This strain is hereinafter referred to as "Bacteria G".

Although the mode of operation of Bacteria G has not definitely been proven and we do not wish to be limited by any theory, it is theorized that its mode of operation is as follows. It has already been experimentally determined that neoplastic cells have antigens which differ from those of non-neoplastic cells. Prior to the present invention, it had not been shown that any neoplasm-specific antigen exists which is common to all neoplastic cells and which distinguishes them from non-neoplastic cells. However, agglutination tests using Bacteria G prove that such an antigen does exist and that the host organism continuously produces antibodies specific to such an antigen. The presence of these natural antibodies is probably a result of the continuing formation of tumour-like cells which are easily eliminated by the normal immune defenses. However, in hosts in which a neoplasm is present and growing, there is something in the serum which is able to prevent the normal antigen/antibody reaction (which would destroy the tumour), this unknown material being hereinafter referred to as "blocking factor".

It is theorized that this blocking factor somehow combines with the neoplasm-antigen and prevents the antibody specific to this antigen from recognizing its presence. If a neoplasm is not recognized, it cannot be destroyed and therefore the neoplastic cells penetrate into normal tissue, break up its organization and are then often able to metastasize. The rate of production of antibodies directed against these neoplastic cells is usually low and is stimulated only by the huge number of cells with potential neoplastic characteristics but which do not produce blocking factor; the fragments of dead neoplastic cells may also contribute to the stimulation of antibody titre. However, even if the antibody titre were large, it could do nothing against a neoplasm producing blocking factor, since the antibodies simply would not recognize the neoplasm.

It is believed that the growth of neoplasms proceeds as follows. First, some cells undergo gradual or rapid transformation, caused by some carcinogenic substance or stimulus, to acquire neoplastic characteristics. Even during the very first phases of the structural transformation, the cell changes or modifies its membrane antigens. At the same time, early in the production of the neoplastic mass, production of blocking factor begins. Once the production of blocking factor is significant, these cells, even if they have not yet assumed all of their cancerous characteristics, are able to circumvent the immunological defenses of the organism. The presence of this blocking factor on the antigen of the neoplastic cells will prevent any contact with immuno-competent cells and, therefore, from the immunological point of view, the cells are considered to be normal. Those cells that have the tumour antigen on their membranes but which are not able to produce sufficient quantities of blocking factor quickly enough would be recognized and rapidly destroyed by the immunological system. This, no doubt, happens very frequently in a normal organism.

The destruction of a neoplastic cell which produces blocking factor may also take place if the transformation has caused the appearance of other strong, specific antigens. Thus, other antibodies may cause the destruction of a neoplastic cell notwithstanding the presence of blocking factor. This is believed to be the reason why the appearance of neoplasms is a relatively rare event.

However, where the antigen is promptly covered with blocking factor, immuno-competent cells will not recognize the neoplastic cells as "non-self" and these cells will, therefore, not be attacked. Accordingly, such neoplastic cells can reach undisturbed a stage in their structural transformation which may soon cause the destruction of the host organism. At this stage, the only limitations on the growth of the neoplastic cells may arise from restricted nourishment - the decline in the health of the organism itself may harm the large and hypo-nourished neoplastic masses. Where this happens, the death of these neoplastic cells leads to the discovery of the tumour antigen and the immuno-competent cells finally begin their attack, but, at this time, there is very little possibility of success; in those rare cases where the immuno-competent cells successfully destroy the neoplastic mass at this stage, there is said to be "spontaneous remission". However, no matter how much antibody is produced, those cells which are still protected by blocking factor will not be

affected. Furthermore, the immune reactions which are now taking place may contribute further to the clinical decline of the patient. Since the antibodies are able to react only against those dead cells which do not produce any blocking factor, and since an enormous number of dead cells will be destroyed, the products of lysis will lead to the progressive but rapid intoxication of the organism, finally leading to death.

We have surprisingly discovered that Bacteria G has the same antigen or, at least, the same antigenic determinant on its cell wall membrane as is present in all neoplastic cells. Thus, the bacterium itself could be regarded as a "neoplastic cell" and is so regarded by the immunological system.

We have discovered, by simple agglutination tests with serum samples from healthy patients, that in substantially all of the sera examined, some measure of agglutination takes place when the serum is contacted with Bacteria G or with an antigenic extract thereof. The extraordinary discovery, however, is that, in testing sera from patients known to have neoplasms, there was no agglutination. The explanation for this discovery lies in the existence of blocking factor. In hosts free from neoplasms, no blocking factor is formed, and thus none is present in the serum. Accordingly, antibodies, which are continually present in the serum of the host, and which are effective against neoplastic cells, will combine with the antigen of Bacteria G and agglutination will occur. On the other hand, in the case of a host infected by a neoplasm, blocking factor will be present in the serum and this blocking factor will mask the antigens on the bacteria, as a result of which no agglutination will occur.

We have determined experimentally that the production of blocking factor is initiated very early in the development of the neoplasm and, therefore, Bacteria G or other bacteria of the genus *Streptococcus* producing a similar or the same antigen, can be used as a very early diagnostic indication of the presence of neoplasms in the host. The extreme importance of such an early indication of the presence of a neoplasm is self-evident.

We have also discovered that the bacteria of the present invention can be used to strip blocking factor from the neoplasm of a host and thus allow the immuno-logical system of the host to invade and destroy the neoplasm. For example, an injection of Bacteria G, or, at least, of the antigenic portion thereof (which is identical to that of the neoplastic cells and which is hereinafter referred to "antigen G"), will eliminate part of the blocking factor by drawing the blocking factor to itself. In other words, the unblocked bacterial antigen G has a greater affinity for blocking factor than does the neoplasm and, accordingly, a proportion of the blocking factor will leave the neoplasm and combine with antigen G from the bacteria. At the same time, the presence of antigen G without blocking factor will greatly stimulate the production of the corresponding antibodies. As a result, after a few days, the titre of antibodies will rise steeply and those neoplastic cells which are left without any blocking factor are rapidly destroyed, first by the humoral immunity and then by the cellular immunity systems. If the neoplasm is not totally destroyed by this first assault, however, the titre of antibody will become progressively lower and the tumour cells will once again produce an excess of blocking factor. At this stage, a second inocculation of antigen G, particularly when it is especially prepared as described hereafter in such a way that it has a greater affinity for blocking factor than for the antibody, again strips a portion of the blocking factor from the neoplastic mass and again leads to a rapid increase in antibody titre. As a result, more neoplastic cells are eliminated. Depending upon the volume of the tumour, its capacity for producing blocking factor and the dose of inocculated antigen G, the neoplasm will be destroyed more or less rapidly. Finally, cicatrized tissue will close the wound and only a few signs of the involution process, which otherwise would have caused the death of host organism will be left.

Accordingly, in its broadest aspect, the invention consists in an antigen produced by a bacterium of the genus *Streptococcus* and characterized by the ability to cause agglutination in the serum of a neoplasm-free patient and not to cause agglutination in the serum of a neoplastic patient.

The invention further consists in a biologically pure culture of a microorganism of the genus *Streptococcus* capable of producing said antigen.

The invention still further consists in killed cells of a bacterium of the genus *Streptococcus* capable of producing said antigen.

The bacterium of the genus *Streptococcus* is preferably Bacteria G, that is *Streptococcus faecalis subspecies G* ATCC 31,290, and the antigen is preferably antigen G (as previously defined), which is produced by Bacteria G.

The cells of the microorganism, Bacteria G, are ovoid, 0.5 to 1.0 μm in diameter, occurring mostly in pairs or short chains and elongate in the direction of the chain. They are non-motile and Gram-positive and endospores are not formed. The nutritional requirements are complex and variable and the microorganism is facultatively anaerobic. Tolerance tests showed growth at 10°C and 45°C, as well as growth in media containing

methylene blue (0.1% w/v in milk), sodium chloride (6.5% w/v) and bile (40% w/v). There was tolerance for growth initiation at pH 9.6 as well as heat tolerance (60°C for 30 minutes). In rich media, such as APT agar, the colonies are larger than usual, and are smooth and entire, rarely pigmented. The microorganism ferments glucose and grows in the presence of 0.04% w/v tellurite, reducing it to tellurium. Gelatin is not hydrolyzed. Growth occurs in the presence of 0.02% w/v sodium azide and a γ-reaction is observed on blood agar. Table 1 below gives the fermentation pattern for this microorganism.

TABLE 1

Glucose	+
Trehalose	+
Lactose	+
Salicin	+
Saccharose	+
Raffinose	+
Maltose	−
Glycerol Aerobic	−
Glycerol Anaerobic	−
Mannitol	−
Sorbitol	−
Arabinose	−
Insulin	−
Citrate	−
Digested Gelatin	−
Bile 10% w/v	+
Bile 40% w/v	+
Litmus Milk	coagulates and makes acid
Methylene Blue 0.1% w/v - Milk	coagulates and makes acid
Growth pH 9.6	+
Arginine Decarboxylase	+
Tellurite 0.04% w/v	+
SF Medium	+

The composition of SF Medium is as follows:

Tryptone	20 g
Glucose	5 g
K_2HPO_4	4 g
KH_2PO_4	1.5 g
NaCl	5 g
Sodium Azide	0.5 g
Bromocresol red	0.032 g
H_2O quant. suff.	1 litre

It can thus be seen that Bacteria G ferments glucose, trehalose, lactose, salicin, saccharose and raffinose within 4 days. It grows on substrates containing 10% w/v and 40% w/v bile. It coagulates and acidifies milk containing litmus and 0.1% w/v methylene blue. It is arganine decarboxylase positive and it grows quickly on SF Medium.

The pattern of sensitivity of the microorganism to certain antibiotics is given in the following Table 2.

TABLE 2

Oleandomycin	++++
Tetracycline	++++
Chloramphenicol	++++
Ampicillin	++++
Riphampin	++++
Terizidone	++−−
Penicillin	++−−
Erythromycin	++−−
Novobiocin	−−−−
Lincomycin	−−−−
Sulfamethoxypyridazine	−−−−
Streptomycin	−−−−
Kanamycin	−−−−
Methicillin	

++++ = sensitive
++−− = slightly sensitive
−−−− = resistant

Based on the above observations, it appears that this microorganism fits the description of *Streptococcus faecalis* appearing in "Bergey's Manual of Determinative Bacteriology", 8th edition, 1974. There are, however, several important properties which differentiate Bacteria G from other microorganisms of the same species. Thus, Bacteria G grows on culture media containing benzopyrene, but which are otherwise the conventional media for growth of microorganisms of the genus *Streptococcus*. The amont of benzopyrene present in the culture medium may be as much as the maximum amount which can be dissolved in it. Benzopyrene, which is an active carcinogen, will prevent the growth of conventional strains of *Streptococcus faecalis*, but does not affect the strain of the present invention, Bacteria G.

Moreover, *Streptococcus faecalis G* undergoes a peculiar growth in the presence of the chemical mutagen, hydroxylamine chloride. At certain intermediate doses of this mutagen, the vitality of the bacteria is protected and growth proceeds. At low and high doses, however, the vitality of the bacteria is substantially reduced; it would have been expected that, as with conventional strains of *Streptococcus faecalis,* all doses of hydroxylamine chloride would inhibit the growth. Moreover, Bacteria G also has the ability to protect paramecia treated with otherwise lethal doses of benzopyrene. Benzopyrene will normally kill paramecia; however, we have discovered that, if they are treated with an extract of Bacteria G, preferably one which has itself been treated with benzopyrene, the paramecia will be completely protected from subsequent treatment with benzopyrene.

Another difference between Bacteria G and conventional strains of *Streptococcus faecalis* is the fact that Bacteria G appears to be immune to the effects of ultraviolet radiation. Although reproduction is at first inhibited, the bacteria recovers in the absence of other inhibiting factors.

The ability of Bacteria G or its antigenic extracts to diagnose the presence or absence of neoplasms by means of a simple agglutination test, even at a very early stage in the growth of the neoplasm, is demonstrated by the following Experiments.

Experiment 1

72 female mice (BALB/C) were divided into seven groups, of which three were used as controls; five of the groups (one of the controls) contained 10 mice and the other two control groups contained 11 mice. Two of the test groups were inoculated intravenously with 3500 infecting doses of Frieund virus (FV), which is known to provoke leukemia, and the other two test groups were inoculated intravenously with a large excess of Rownson-Parr virus (RPV), which is known to produce splenic lymphoma. 15 days after inoculation, the mice were killed and their plasma removed.

The plasma was separated from erythrocytes by centrifuging and was kept at 3°C until use. To each of a series of serially diluted plasma samples was added 0.01 cc of a Bacteria G suspension having an optical density of 0.400, read at 420 nm. The plasma containing the Bacteria G was then incubated for 24 hours at 37°C, and then examined for the presence or absence of agglutination. The spleen weight of each of the animals was also measured.

The relationship between the spleen weight and the presence or absence of agglutination in the mice is shown in Tables 3, 4 and 5.

TABLE 3

Relation between spleen weight and reaction of agglutination in mice (BALB/C) infected with leukemia FV

Spleen Weight (mg)	Reaction after Incubation for 24 hours at 37 °C
284	Agglutination
892	Non Agglutination
2026	" "
971	" "
626	" "
553	" "
475	" "
1990	" "
524	" "
387	" "
605	" "
1133	" "
775	" "
422	" "
566	" "
760	" "
833	" "
741	" "
353	" "
673	" "

TABLE 4

Relation between spleen weight and reaction of agglutination in mice (BALB/C) infected by splenic lymphomata RPV

Spleen Weight (mg)	Reaction after Incubation for 24 hours at 37 °C
250	Non Agglutination
328	" "
477	" "
353	" "
320	" "
363	" "
313	" "
275	" "
227	" "
386	" "
452	" "
417	" "
413	" "
153	Agglutination
265	Non Agglutination
202	" "
276	" "
355	" "
172	Agglutination
240	Non Agglutination

TABLE 5

Relation between spleen weight and reaction of agglutination in mice (BALB/C) of the control group.

Spleen Weight (mg)	Reaction after Incubation for 24 hours at 37 °C
123	Agglutination
171	"
145	"
109	"
106	"
112	"
106	"
101	"
111	"
131	"
126	"
100	"
112	"
148	"
140	"
145	"
94	"
177	"
141	"
147	"
89	Non Agglutination
178	Agglutination
95	"
138	"
148	"
178	"
118	"
101	"
116	"
120	"
130	"
147	"

These results may be summarized as follows. Only one case of non-agglutination was found in the control mice. The groups infected with FV gave only one case of agglutination. The groups infected with RPV gave only two cases of agglutination. An increase in the spleen weight of the inoculated mice, determined soon after death, was taken to be an indication that infection had occurred. In the case of RPV, it is known that, soon after infection, the spleen increases in size and this continues for 15 - 20 days, after which it returns to normal and does not exhibit any neoplastic lesions until 6 - 8 months after infection. It is noted that the only case of non-agglutination in the control group showed a very low spleen weight.

This experiment demonstrates the highly significant fact that, in mice infected with either FV or RPV, blocking factor apparently is present in the serum only 3 or 4 days after inoculation of the virus. This is 6 or 7 months before the presence of splenic lymphoma could hitherto have been determined clinically. Thus, very early diagnosis is possible using the method of the present invention.

Thus, the invention further consists in a method of detecting the presence of blocking factor (as herein defined) in plasma by incubating the plasma with Bacteria G or with an antigen G-containing extract thereof.

Experiment 2

Tests similar to those described in Experiment 1 were conducted on the sera of human patients, some of whom had histologically diagnosed neoplastic conditions, and some of

whom had no previous indication of neoplasms and were thus used as controls. The results are shown in Table 6. It should be noted that these were blind tests, in that the experimenter did not known the identity of the serum at the time of the agglutination test. Some of the sera used in these experiments were also tested for agglutination with a suspension of a conventional strain of *Streptococcus faecalis*. The results are shown in Table 7.

TABLE 6

Sera of patients with ascertained neoplasia tested by bacterial G suspension

Histologic Diagnosis	Reaction of Agglutination Dilutions 1/2	1/4	1/8
Adenocarcinoma	–	–	–
Epithelioma	–	–	–
Neck Carcinoma	–	–	–
Carcinoma	–	–	–
Carcinoma	–	–	–
Adenoma	–	–	±
Lipoma	–	–	–
II Stadium Cancer	–	–	±
Carcinoma	±	–	–
Adenocarcinoma	–	–	–
Adenocarcinoma	–	–	–
Lipoma	–	–	±
Carcinoma	++++	+++	+++
Adenocarcinoma-Diffused Metastases	±	±	–
Rectum Adenocarcinoma	±	–	–
Prostatic Carcinoma	–	–	–
Carcinoma	±	–	–
Colon Adenocarcinoma	–	–	–
Colon Carcinoma	–	–	–
Mamma Carcinoma	–	–	±
Metastatic Carcinoma	++++	++	++
Metastatic Adenocarcinoma	±	±	±
Myeloid Leukaemia	±	–	–
Myeloid Leukaemia			

TABLE 6 (Continued)

Histologic Diagnosis	Reaction of Agglutination Dilutions 1/2	1/4	1/8
Mamma Carcinoma with Diffused Metastases	++++	++	++
Epithelioma	−	−	−
Lymphoma	±	−	−
Neck Carcinoma *	±	±	±
Adenoma *	±	−	±
Stomach Cancer *	−	−	−
Carcinoma *	−	−	−
Ulcerating Carcinoma *	±	−	−
Bony Metastases by Prostatic Carcinoma *	++++	+++	++
Lymphoma *	±	−	−
Portio Carcinoma *	−	−	−
Carcinoma *	−	−	−
Carcinoma with Bony Metastases*	+++	++	++
I Stadium Adenocarcinoma	±	±	±
Rectum Adenocarcinoma	±	−	−
Metastases by Mamma Carcinoma	++++	+++	+++
Hepatic Carcinoma *	±	±	±
Epithelioma	±	−	−
Lymphoma	±	−	−
Stomach Cancer	−	−	−
Carcinoma	±	−	±
Adenocarcinoma	−	−	−
Adenoma	±	±	+
Mamma Carcinoma	−	−	−
Diffused Metastases by Mamma Cancer	±	+	+
Prostatic Carcinoma	−	±	−
Adenocarcinoma	−	−	−
Metastatic Adenocarcinoma	++++	++++	+++
Control serum from non-cancer patients 56 cases	++++ or +++	+++ or ++	++
3 cases	+	+	±
1 case	+	±	±

* These sera was also tested with *Streptococcus faecalis* (see table 7)

−	=	non-agglutination
±	=	doubtful
+	=	agglutination
+ − ++++	=	relative degree of agglutination.

TABLE 7

Agglutination reaction with Bacterium G or Streptococcus faecalis

Histologic Diagnosis	Dilutions of the Bacterium G Suspension			Dilutions of the *Streptococcus Faecalis* Suspension		
	1/2	1/4	1/8	1/2	1/4	1/8
Neck Carcinoma	±	±	±	++	++	+±
Adenoma	±	−	±	++++	+++	++
Stomach Cancer	−	−	−	+++	+++	++
Carcinoma	−	−	−	±	−	−
Ulcerating Carcinoma	±	−	−	+++	+++	+±
Bony Metastases by Prostatic Carcinoma	++++	+++	++	++	++	+
Lymphoma	±	−	−	+	+	±
Portio Carcinoma	−	−	−	+++	+++	++
Carcinoma	−	−	−	++	+	±
Carcinoma with Bony Metastases	+++	++	++	++	++	+
Hepatic Carcinoma	±	±	+	+++	++	+
Control serum from non-cancer patients 20 cases	++++ or +++	+++ or ++	++	++++ or +++	+++ or ++	++

It can be seen from Table 6 that no agglutination or doubtful agglutination occurred in 45 of the 52 cases in which there was a histological diagnosis of a neoplastic condition. The seven cases which gave substantial agglutination notwithstanding the presence of neoplasms were all cases in which the neoplasm was very far advanced and metastasis had already occurred. It is theorized that these neoplasms were so far advanced that there was no longer sufficient blocking factor in the serum to prevent agglutination. In a clinical situation, however, the diagnostic test provided by the present invention would not be necessary for such patients, since, in these cases, the neoplasm is so far advanced that there is no question of its existence.

In the control group, the doubtful cases are probably due either to a very low antibody titre in this particular patient or the possibility that the patient has a hitherto undiagnosed neoplasm.

Comparison of the results reported in Table 6 with those using a conventional strain of *Streptococcus faecalis* reported in Table 7 shows that, using the conventional strain, agglutination occurred in substantially all cases, notwithstanding the presence or absence of neoplasms.

In both of the above experiments, the bacteria was cultured for 24 hours at 37° in Tryptic Soy Broth (TSB), a product of Difco Inc.. The composition of TSB is as follows:

Tryptone	17 g
Soytone	3 g
NaCl	5 g
H_2Q quant. suff.	1 litre.

The cells were then centrifuged and washed with a 0.45% w/v aqueous sodium chloride solution. The cells were then diluted in a 0.45% w/v aqueous sodium chloride solution to an extent sufficient to achieve an optical density reading of 0.400 at 420 nm. The solution was then refrigerated at 5°C until use. The conventional strain of *Streptococcus faecalis* used in these experiments is the strain available from the American Type Culture Collection under the accession No. ATCC 8043.

It can be seen from the above experiments that Bacteria G, or at least the antigenic portion thereof which we refer to as "antigen G", on tests with known neoplastic and

non-neoplastic sera shows the ability to link both blocking factor (in known neoplastic sera) and antibody (in known non-neoplastic sera). Since this property is a property of antigen in the bacteria, either the antigen itself, the live Bacteria G, killed Bacteria G or any portion of the bacteria showing this antigenic activity can be used. Live Bacteria G are not harmful to humans.

The concentration of bacteria or antigen to be used is not critical, provided that agglutination can be detected. In the present specification, we measure concentration of the bacteria by means of optical density of a suspension of bacteria in any physiological solution at 420 nm. Below an optical density of 0.200, it is very difficult to detect agglutination at normal magnification. Above 0.500, the bacteria are themselves so dense as to make it difficult to detect agglutination. Accordingly, although any concentration at which agglutination may be detected can be used, preferred concentrations are between 0.200 and 0.500 optical density at 420 nm; most preferably, we use a concentration corresponding to an optical density of 0.400. The bacteria may be present in any physiological solution.

Although the above experiments have been carried out with live bacteria, identical results are achieved with killed bacteria; however, if the bacteria are to be killed, the reagent used to kill them should not be one which will affect the ability of the antigen G to link with blocking factor and antibody. For example, strong oxidizing agents can affect the ability to link with blocking factor and these should not, therefore, be used. We have found that the most convenient reagent for killing bacteria is phenol.

It is desirable that the antigen G-containing material (e.g. the bacteria) used in the agglutination test should be able to agglutinate within 24 hours at body temperature. Since blocking factor will be destroyed if kept too long, the results will not be significant if it takes over 24 hours for the agglutination to occur. Again, however, simple and routine testing of any given antigen G-containing material on non-neoplastic sera will ascertain whether that material can cause agglutination within 24 hours.

The experiments above demonstrate two important features of the present invention. First, the presence of neoplastic growth can be detected at a very early stage, much earlier than has hitherto been possible. At the very least, the diagnostic method of the invention can be used to confirm a doubtful diagnosis or to warn of the possibility of the presence of a neoplasm. Second, since common results were achieved with a large number of different types of neoplasm, the experiments provide evidence for the hypothesis that there is an antigen common to all neoplasms.

Bacteria G is thus significant not only because it bears an antigen which is antigenically similar to that common to all neoplasms, but also because it apparently does not produce any blocking factor and, in fact, has the ability to cause blocking factor which is already linked to a neoplasm to leave the neoplasm and become linked to the bacteria. These properties enable the bacteria to be used in the treatment of neoplasms, as verified by the following *in vitro* experiments.

Experiment 3

Cultures of Hela and KB cancer cells, six or seven days old, were prepared on slides and then cultured in test tubes of Medium 199. The Medium 199 was then poured out of the test tubes and Eagle's Medium containing a suspension of Bacteria G was then added. A large excess of bacterial cells over cancer cells was used. After leaving the bacteria and cancer cells in contact for approximately 4 hours, the bacteria were removed by pouring off the medium. A solution of antibodies from human serum or human serum itself was then added, along with a complementary system (Sclavo). After contact with the serum and complement, it was seen that the cancer cells which had been in contact with the Bacteria G had been destroyed by lysis. Control cells, which had not been treated with the bacteria, were not affected by the antibodies or the serum. This experiment demonstrates not only that blocking factor is apparently used by the neoplastic cells themselves but also that Bacteria G can remove the blocking factor from the neoplastic cells.

In order to use Bacteria G in the treatment of neoplasms by stripping the neoplasms of their blocking factor and thus allowing their destruction by the natural antibodies, optimum results are achieved if the bacteria used have the greatest possible ability to link with blocking factor (affinity for blocking factor) while, at the same time, they have the lowest possible ability to link with antibody (affinity for antibody). The injection of such bacteria would cause the largest possible amount of blocking factor to be stripped from the neoplasm and the smallest possible amount of antibody to be wasted by linkage to the non-pathogenic bacteria. We have determined experimentally that the ability of bacteria to link with either blocking factor or antibody varies depending upon the method of culture of the bacteria and that, by carefully standardizing the conditions of growth, optimum conditions can be obtained. It should be understood, however, that, regardless of the conditions of growth, useful results can be achieved by treatment with Bacteria G or an

antigenic extract thereof, particularly upon the first treatment.

In tests on rats with natural tumours (mostly mamma adeno-cancer) as well as on rats inoculated with Walker carceno-sarcoma 256, we observed that, after 2 or 3 treatments with Bacteria G in doses of 1.5 units of optical density at 420 nm (injected subcutaneously at the rate of 1 cc per rat) the tumour was totally destroyed in about 50% of the treated animals. In the remaining animals, large tumours were present and these had already reached an advanced stage; however, necrosis of the tumour with serious bloody inflammation of the surrounding tissue and a reduction of the tumour mass by about one fifth of its initial mass, was noted. These results, however, were attributable to the first treatment and no further significant results were noticed on second or third treatment. It is theorized that the reason why the outstanding results did not continue in the second and third treatment arises from the ability of Bacteria G to link with the antibodies. In the first treatment, the antibody titre is very low and thus a large dose of bacteria will cause a large amount of blocking factor to be stripped from the neoplasm, before the bacteria is destroyed by the antibodies. After the first treatment, however, the antibody titre will drastically increase and thus the effect of further treatments is much reduced because the increased titre of antibody will destroy the bacteria before it has a chance to strip a substantial amount of blocking factor from the neoplasm.

In order to determine the various degrees of ability to link with blocking factor and antibody during the various phases of growth of the bacteria, the following experiment was conducted.

Experiment 4

A large number of mice were inoculated subcutaneously with ascitic liquid from an Ehrlich tumour and, five days after inoculation, those animals with evidence of an increased nodule not exceeding 3 mm diameter were selected. A total of 300 mice were selected. These were divided into 5 groups of sixty mice each. In order to avoid the effects of low threshold or high threshold with different suspensions of bacteria, each group of 60 mice was divided into 6 sub-groups of 10 mice each and these sub-groups, although being treated with the same type of suspension, received six different graduated doses of the same. One of the five groups served as a control group and thus did not undergo any treatment. The other four groups were each treated with a suspension of a different age, i.e. a 1-day culture, a 3-day culture, a 5-day culture and a 7-day culture. The different doses were expressed in units of optical density at 420 nm, i.e. 0.050, 0.100, 0.200, 0.400, 0.800 and 1.600. The bacterial suspension was administered in a volume of 0.3 cc to each mouse by subcutaneous inoculation into the back of the mouse. The first treatment was begun on the fifth day of growth of the transplanted neoplastic mass. The second treatment was given 11 days after the first treatment (the sixteenth day of growth) and the third treatment was given 11 days after (the twentyseventh day of growth). 35 days after the initial transplantation of the neoplastic mass, the mice were examined to determine the ability of the bacteria to produce immunization, their ability to stabilize tumour growth and their ability to cause regression. The results are shown in Table 9.

TABLE 9

Doses	1 Day Culture			3 Day Culture			5 Day Culture			7 Day Culture		
	I treatment	II treatment	III treatm.	I treatm.	II treatm.	III treatm.	I treatm.	II treatm.	III treatm.	I treatm.	II treatm.	III treatm.
0.3 ml O.D. = 0.050 at 420nm	/	/	/	/	/	/	/	/	/	/	/	/
0.3 ml O.D. = 0.100 at 420nm	stasis	/	/	stasis	/	/	/	/	/	/	/	/
0.3 ml O.D. = 0.200 at 420nm	stasis	/	/	stasis	stasis	survival greater than the control in 70% of mice	/	/	/	/	/	/
0.3 ml O.D. = 0.400 at 420nm	stasis	/	/	stasis	/	survival greater than the control in 20% of the mice	/	/	/	stasis	regres-sion in some mice	regres-sion in 90% of the mice
0.3 ml O.D. = 0.800 at 420nm	/	/	/	/	/	/	stasis	stasis	/	stasis	regres-sion in some mice	regres-sion in 90% of the mice
0.3 ml O.D. = 1.600 at 420nm	/	/	/	/	/	/	stasis	stasis	/	/	/	/

Note: / = like the control group

All mice of the control group had died after 28 - 30 days. In the table, regression means elimination of the tumour.

The results in Table 9 may be summarized as follows. The group treated with a suspension prepared from a culture incubated for 1 day, as compared with the control, showed tumour stasis at doses of 0.100, 0.200 and 0.400 units after the first inoculation only. However, after the second inoculation, there was an increase in mortality, with ulceration of the tumour mass. Thus, this group, instead of recovering, worsened and presented a higher index of mortality than did the control mice.

The group treated with a suspension prepared from the 3-day culture did not present any substantial difference from the group treated with the 1-day culture. The dose which gave the highest survival was 0.200.

The group treated with suspensions of bacteria incubated for 5 days showed tumour stasis at doses of 0.800 and 1.600 units after the first treatment. However, upon the second treatment, a sudden increase in mortality was noted.

The group of mice treated with a 7-day culture gave quite positive results, with a rate of survival superior to that of the control group and with regression of the tumour mass at doses of 0.400 and 0.800 units. In practice, in this last group, only three treatments were necessary to block continued growth and to destroy the tumours. With this 7-day culture at dose rates of 0.400 and 0.800 units, complete elimination of the tumour in 90% of the mice was achieved, which is a surprising and significant result. In fact, the only mice which had died within 28 days of the transplantation of the neoplastic mass were the ones treated with doses of 0.050 units. In the other animals, those which did not exhibit regression of the tumour showed complete stasis and in almost all of the mice the tumour was rather hard and hypotrophic and, it seemed, also calcified. In the control mice, the tumour never became particularly hard. Before achieving complete regression of the tumour, a part of the neoplastic mass often sloughed off, causing a kind of abscess to open. However, at the end of the trial period, cicatrization of the tissues affected by the tumour process was complete and could only be noted with great difficulty.

The conclusions which can be drawn from this experiment are that cultures of Bacteria G grown at 37°C on TSB produce an antigen whose affinity for blocking factor and/or antibodies is very variable, depending upon the age of the culture. The results from this experiment show that the optimum culture is one produced by 6 - 7 days incubation on TSB at 37°C.

Even better results are obtained with more constantly reproducable optimum dose rates of the culture, when the culture of Bacteria G is maintained under conditions of controlled aerobic growth. This controlled aerobic growth, which is hereafter referred to as "hypoxia", occurs when, after inoculation, the culture is sealed in an airtight vessel and maintained without shaking until use. This contrasts with an oxgenated culture, where the bacteria is cultivated in a free supply of air, with shaking. The difference between oxgenated cultures and those grown in hypoxia can be seen from the following experiment.

Experiment 5

180 mice were inoculated subcutaneously with ascitic liquid from Ehrlich tumours, as in the previous experiment. They were then divided into three groups of 60 mice and each of these groups was divided into 6 sub-groups. As in the previous experiment, each sub-group received a different dose of Bacteria G. The first group received doses of Bacteria G grown in oxygenated cultures; the second group received doses of Bacteria G grown in hypoxia; the third group were used as a control and did not receive any treatment with Bacteria G. The first treatment was made 5 days afer transplantation of the neoplastic mass; the second treatment was given 11 days later; and the third treatment was given 11 days after the second treatment. The results are shown in Tables 10 and 11. In each case, the cultures of Bacteria G used were 6 days old.

TABLE 10

Mice treated with a 6-day Bacterium G grown in aerated medium (O.D. at 192 nm = 0.85)

Doses of 0.3ml (O.D. at 420nm)	II Treatment after 11 Days	III Treatment after Second 11 Days	Results after the III Treatment
0.050	/	/	/
0.100	/	/	/
0.200	Stasis in 60% of mice	Stasis in 60% of mice	Stasis. The remaining 40% of mice like control
0.400	Stasis in 60% of mice	Stasis in 60% of mice	Stasis. The remaining 40% of mice like control
0.800	Stasis in 70% of mice	Stasis in 70% of mice	Stasis. The remaining 30% of mice like control
1.600	Stasis	Stasis	Stasis

Note: / = like the control group

TABLE 11

Mice treated with a 6-day Bacterium G grown in hypoxia. (O.D. at 192 nm = 1.4)

Doses of 0.3ml (O.D. at 420nm)	II Treatment after 11 days	III Treatment after second 11 days	Results after the III treatment
0.050	/	/	/
0.100	Stasis	Stasis	Stasis
0.200	Regression in 50% of mice	Regression in 60% of mice	Tumor reduced in 40% of mice; Regression in 60% of mice
0.400	Regression in 80% of mice	Regression	Regression
0.800	Regression in 80% of mice	Regression	Regression
1.600	Stasis	Stasis	Stasis

/ = like the control group
Regression = Complete elimination of tumour

Although a remarkable improvement in the condition of the group of mice treated with oxgenated cultures, compared with the control groups, was noted, only a relatively few isolated regressions occurred and these had no apparent connection with specific doses. On the other hand, the results achieved in the group treated with cultures grown in hypoxia were even more remarkable. Complete regression of the tumour mass in mice treated with doses of 0.200, 0.400 and 0.800 units was achieved; stasis was achieved with doses of 0.100 and 1.600 units. No protection was noted with doses of 0.050 units.

After the first treatment with the cultures of Bacteria G, microscopic inflammation of the tumour mass appeared; this occurred later with the cultures grown in hypoxia than with the oxygenated cultures. It is thought that this is caused by the inferior affinity of the cultures grown in hypoxia for antibodies; thus, the required dose for the first inflammatory observation is higher with these cultures. On the second treatment, the animals treated with successive doses to the ones which achieved the first inflammatory dose gave a better reply. Later there were no differences in the original doses. In the animals achieving tumour stasis, there is no evidence of any worsening of their general condition at a microscopic level and these animals behaved and looked quite different from the control animals: they cleaned themselves, their hair was in good order and they looked quite normal.

We have found that one way of determining the relative affinities of a culture towards blocking factor and towards the antibodies is to take a suspension of bacteria having an optical density of 0.07 at 420 nm and then determine its optical density at 192 nm. Surprisingly, the greater the optical density at 192 nm (at this particular concentration), the greater will be the relative affinity of the bacteria for blocking factor and therefore the better are the bacteria suited for clinical use.

It should be noted that, in Experiment 5 the optical density at 192 nm of a suspension having an optical density of 0.07 at 420 nm was 1.4 for bacteria grown in hypoxia and 0.85 for bacteria grown in an oxygenated culture. It is therefore theorized that good results will be obtained when the optical density at 192 nm of a bacterial suspension having an optical density at 420 nm of 0.07 is at least 0.70 and that better results will be obtained when the optical density is greater than 0.90. Accordingly, it is more preferred that this optical density should be greater than 1.0 and most preferred that the optical density be greater than 1.2 .

It is expected that the optimum age of a culture and the preferred method of growth may differ, depending upon the particular culture medium used; however, measurement of optical density at 192 nm can give a quick indication of the type of results which can be expected and, therefore, of the optimum culture to be used. Table 12 shows optical density (OD) values at 192 nm for various culture media and several culture ages; each suspension had an optical density of 0.07 at 420 nm and, in each case, the bacteria were grown in hypoxia.

TABLE 12

	Age of the culture		
Culture Medium	40 hours	4 days	7 days
TSBG	0.700	1.500	1.560
TSAG	0.550	0.680	0.800
MH	0.750	0.730	1.270
SF	0.340	1.580*	1.700*

* Very high values owing to the Bromocresol Red in the cultural medium

The culture media used were as follows:

TSBG:	
Triptone	17 g
Soytone	3 g
Glucose	2.5 g
NaCl	5 g
K_2HPO_4	2.5 g
H_2O quant. suff.	1 litre
TSAG:	
Triptone	17 g
Soytone	3 g
Glucose	2.5 g
NaCl	5 g
K_2HPO_4	2.5 g
Agar	20 g
H_2O quant. suff.	1 litre
Mueller Hinton:	
Beef Extract	3 g
Bacto Casimino Acids	17.5 g
Starch	1.5 g
H_2O quant. suff.	1 litre

SF medium is as previously described

It should be understood that, although the age of the culture and the way in which the cultivation is carried out has an effect upon the bacteria produced, this is only significant in determining the relative effect of treatment with Bacteria G. Even 1-day cultures grown in oxygenated medium give useful results if only one treatment is required and such cultures therefore form part of the present invention. To a patient having a neoplasm, even stasis is a significant and important improvement.

Experiment 6

Groups of 25 test mice (CDF/1) were inoculated intraperitoneally with about 10^6 cells of leukaemia L1210. The mice were chosen to have a body weight between 25 and 30 g. One of the groups of mice was chosen as a control group and received no treatment. Details of mortality and average weight increase are shown in Table 13. Another of the groups of mice was treated with a suspension containing the known bacteria *Streptococcus faecalis* ATCC 8043. Each mouse received three intramuscular injections of a 0.2 cc suspension of this bacterium having an optical density of 1.200 at 420 nm. The results are shown in Table 14. The third group received three intramuscular injections each of 0.2 cc of a suspension of Bacteria G having an optical density of 1.200 at 420 nm. The results are reported in Table 15.

The suspensions of both strains of *Streptococcus faecalis* were produced by growth on a medium of TSB at 37°C for 6 days in hypoxia. It can be seen from Tables 13 and 14 that 100% mortality was reached within 9 days with the control group and within 11 days with the group treated with *Streptococcus faecalis* ATCC 8043. However, 100% mortality was not reached with the group treated with Bacteria G, even after 25 days.

TABLE 13

Day	Treatments	Mortality %	Weight Increase (average)
0	=	=	=
1	=	=	=
2	=	=	0.4 gr
3	=	=	1 gr
4	=	=	1.5 gr
5	=	=	1.8 gr
6	=	10%	2.5 gr
7	=	30%	=
8	=	80%	=
9	=	100%	=

TABLE 14

Day	Treatments	Mortality %	Weight Increase (average)
0	1°	=	=
1	=	=	=
2	=	=	0.8
3	=	=	0.8
4	=	=	1.5
5	2°	=	2
6	=	20%	2.8
7	=	30%	=
8	=	70%	=
9	=	80%	=
10	3°	90%	=
11	=	100%	=

TABLE 15

Day	Treatments	Mortality %	Weight Increase (average)
0	1°	=	=
1		=	0.8 gr
2		=	0.6 gr
3		=	0.4 gr
4		=	=
5	2°	=	=
6		=	=
7		=	0.4 gr
8		=	0.6 gr
9		=	0.8 gr
10	3°	20%	0.8 gr
11		=	0.8 gr
12		30%	
13		=	
14		=	
15	4°	=	
16		=	
17		40%	
18		=	
19		=	
20		60%	
21		=	
22		=	
23		=	
24		=	
25		=	

From the results given above, it can be seen that Bacteria G and antigenic extracts thereof can be used to treat neoplasms in human patients. Accordingly, the invention further provides a pharmaceutical composition comprising Bacteria G or an antigen G-containing extract thereof in admixture with a pharmaceutically acceptable carrier or diluent.

Any method of administration commonly used for pharmaceutical compositions may be used with the composition of the present invention, depending upon the nature and location of the neoplasm to be treated. Oral or parenteral administration is preferred and intravenous administration is more preferred, although administration may also be by the intramuscular route. The dosage should be determined on a case-by-case basis (depending upon the severity of the symptoms and the mode of administration). It is, however, important that the quantity of bacteria injected should be sufficient to link with a sufficient quantity of blocking factor to allow the antibodies of the host to attack the neoplastic cells. The quantity should not, however, be so great as to cause linkage with substantial quantities of the antibodies produced. As an example, an intravenous injection of 2 cc of Bacteria G in suspension at a concentration giving an optical density of 2.0 at 420 nm may be given. If any signs of anaphylatic shock appear, this indicates that the initial dose was too great and more time should be left between treatments. The second dose should not be given until the antibody titre in the serum of the patient (which will have risen substantially as a result of the first injection) decreases to a constant level. This may take as much as 11 to 20 days, generally 11 days, although, as discussed hereafter, doses may be given at somewhat more frequent intervals. If no sign of anaphylatic shock occurs after the first injection, then the second injection should be the same dose. Treatment should be continued until the neoplastic condition is corrected.

More detailed information on the modes of administration is as follows:

Subcutaneous administration

This is suitable in the case of very large neoplasms. It allows the administered Bacteria G to remain at the affected site for a relatively long time and, moreover, because of the low exchange rate of the blocking factor, it minimizes the possibility of extensive necrosis. The dosage should be chosen having regard to the seriousness of the pathological picture. It is advisable to start by administering from 1 to 2 cc of a suspension of Bacteria G having an

optical density at 420 nm of 1.4. This dose should be administered on two successive days, after which there should be a break of 6 or 7 days, followed by administration on two successive days; this pattern of administration should continue until the neoplasm has been destroyed.

Intramuscular injection

This is suitable in the case of neoplasms just starting and, in any case, not affecting well-vascularized organs, because of the hazard of haemorrhage because of necrosis of the neoplasm. It allows for good exchange of blocking factor and fast elimination of the Bacteria G. The dose can be varied over a very wide range, generally from 1 to 10 cc of a suspension having an optical density at 420 nm of 1.400. The dose is preferably administered on two or three successive days, followed by a break of 6 or 7 days, then administration for 2 or 3 successive days, again followed by a 6 or 7 day break. The preferred plan of administration is the same as for subcutaneous administration.

Intravenous injection

This allows very good exchange of blocking factor but has some disadvantages, principally because the Bacteria G is eliminated too fast. It is, therefore, best to use this mode of administration only to speed up the appearance of the primary immunological response. In any case, the dose of bacterium G administered intravenously should never exceed the limit of a hypothetical dilution in the blood corresponding to an optical density of 0.03 at 420 nm, because of the hazard of immunitary block. For an adult patient having a body weight of 65 - 70 kg, this limit is reached with about 2 doses each of 10 cc of a Bacteria G suspension having an optical density at 420 nm of 1.000.

Administration per os

Doses have to be increased owing to losses by dispersion in the chyme and chyle. Nevertheless, this is the most convenient mode of administration. We prefer that the Bacteria G should be lyophilized and placed in capsules resistant to gastric juices to allow the capsules to pass undigested through the gastric system. If the Bacteria G are alive when administered, they may proliferate in the intestines, which can provide a useful extra dose of bacteria. The plan of administration is preferably the same as for intramuscular administration. The dose preferably ranges from 0.01 to 0.1 g of lyophilized Bacteria G.

Rectal administration

A suspension of Bacteria G can be administered rectally. It has the same advantages as administration *per os* and, moreover, does not run the risk of enzymatic degradation. The dose is preferably administered after evacuation of excrement and in an amount of from 10 to 20 cc. The plan of administration is preferably the same as for intramuscular administration.

Topical administration

This mode of administration is only subsidiary and preferably should only be used in association with other modes of administration and with the same plan of administration as that other mode. The dose depends upon the extent of the lesion although a relatively small amount of Bacteria G suspension (1 - 5 cc of optical density 1.000 at 420 nm) is preferably used. The Bacteria G may be formulated with any suitable carrier or diluent conventional for topical preparations.

In general, any carrier or diluent conventionally used for pharmaceutical preparations may be used with Bacteria G or with its antigenic extract and the particular carrier or diluent will be chosen having regard to which of the above modes of administration is employed. However, carriers or diluents having a strongly oxidizing or reducing action are not recommended. We prefer that the appropriate diluent should be added to the Bacteria G (lyophilized or frozen at −20°C) a short time before administration. Preferred formulations are:

live Bacteria G plus isotonic saline (0.9 % w/v NaCl)
live Bacteria G plus physiological saline without glucose
live Bacteria G plus phosphate buffer
live Bacteria G plus 0.9 % w/v NaCl plus 0.5 % w/v phenol
lyphilized live Bacteria G in a gastric-resistant capsule
suspensions in distilled water.

In the formulations containing phenol, the phenol kills the bacteria and we prefer that this should be prepared 1 hour before use. In the case of suspensions in distilled water, it is recommended that the suspension should be used immediately after it has been prepared. In other cases it is recommended that the preparation should be used soon after it has been

prepared.

In all of the experiments described above, live bacteria were used; however, on repeating the experiments with killed bacterial cells, substantially the same results were achieved.

WHAT WE CLAIM IS:

1. An antigen produced by a bacterium of the genus *Streptococcus* and characterized by the ability to cause agglutination in the serum of a neoplasm-free patient and not to cause agglutination in the serum of a neoplastic patient.

2. An antigen according to Claim 1, in which said bacterium is of the subspecies *Streptococcus faecalis subspecies G* ATCC 31290.

3. A biologically pure culture of a microorganism of the genus *Streptococcus* capable of producing an antigen according to Claim 1.

4. A culture according to Claim 3, in which said microorganism is *Streptococcus faecalis subspecies G* ATCC 31290.

5. Killed cells of a bacterium of the genus *Streptococcus* capable of producing an antigen according to Claim 1.

6. Killed cells according to Claim 5, in which said bacterium is *Streptococcus faecalis subspecies G* ATCC 31290.

7. A pharmaceutical composition comprising Bacteria G (as hereinbefore defined) or an antigen G-containing extract thereof (as hereinbefore defined) in admixture with a pharmaceutically acceptable carrier or diluent.

8. A composition according to Claim 7, in which the Bacteria G is such that a washed physiological solution of cells thereof diluted to a concentration having an optical density of 0.07 at 420 nm has an optical density of at least 0.70 at 192 nm.

9. A composition according to Claim 8, in which said optical density at 192 nm is at least 0.90.

10. A composition according to Claim 9, in which said optical density at 192 nm is at least 1.0.

11. A composition according to Claim 10, in which said optical density at 192 nm is at least 1.2.

12. A composition according to any one of Claims 7 to 11, formulated for intravenous or intramuscular injection.

13. A composition according to any one of Claims 7 to 11, formulated for oral administration.

14. A composition according to Claim 13, in a capsule of a material resistant to gastric secretions.

15. A method of detecting the presence of blocking factor (as herein defined) in plasma by incubating the plasma with Bacteria G or with an antigen G-containing extract thereof.

16. A method according to Claim 15, in which said Bacteria G has the characteristics specified in any one of Claims 8, 9, 10 and 11.

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